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Precision CO_2 Control in CHO-Based Bioprocessing With the Biostat® B-DCU

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Abstract

Precise control of dissolved carbon dioxide (dCO_2) is critical for optimizing cell viability, growth, and product quality in biopharmaceutical production. This study demonstrates the Biostat® B-DCU's advanced capabilities in implementing a robust dCO_2 control strategy for a CHO-based IgG production process in a 2 L bioreactor. Utilizing the CO_2NTROL optical sensor and Sartorius SCADA software, the system achieved stable dCO_2 regulation, minimal interference with pH and dissolved oxygen control, and did not significantly affect cell productivity or volumetric productivity.

The results highlight the B-DCU's precision and scalability, establishing the system as an essential tool for bioprocess engineers seeking to replicate large-scale conditions at bench scale while ensuring high product yields.

Introduction

Dissolved CO_2 significantly modulates cellular metabolism, intracellular pH, and osmolality, compromising cell viability and biopharmaceutical product quality attributes, such as IgG glycosylation.[1] Elevated dCO_2 levels can cause metabolic stress, inhibit growth, and alter protein quality, with cell-line-specific responses observed at high concentrations (e.g., 15% saturation or 120 mm Hg).[2] The Biostat® B-DCU, equipped with advanced process analytical technology (PAT), enables real-time dCO_2 monitoring and control, ensuring optimal process conditions.

This study evaluates a dCO_2 control strategy in a 2 L CHO-based fed-batch process, specifically examining control stability, parameter interactions, and productivity. The results demonstrate the Biostat® B-DCU's efficacy in addressing large-scale bioprocessing challenges at the bench scale.

Materials and Methods

Cell Culture

CHO-IgG1 cells were cultured in HyClone™ SFM4CHO (Cytiva Europe GmbH) with 4 mM glutamine, 7.5 g/L glucose, 2.2 g/L sodium bicarbonate, and 0.1% (w/v) Pluronic F68, at pH 7.2 – 7.4. Cells were seeded at $2 - 4 \times 10^5$ cells/mL, passaged every 48 – 72 h, and incubated at 37 °C, 5% CO_2, 85% relative humidity, and 140 rpm. The seed train started at 4×10^5 cells/mL in pseudo-perfusion mode using non-baffled PETG shake flasks (Thermo Fisher Scientific). G2-phase cells were enriched via centrifugation (80 × g for 2 min) and resuspended in a 1:100 conditioned | fresh medium mix with 5 mM glutamine, scaling from 50 to 200 mL. A final medium exchange achieved an ultrahigh density of 1×10^8 cells/mL for reactor inoculation.

Bioprocess Setup

A fed-batch process was conducted in a Biostat® B-DCU 2 L stirred-tank Univessel® Glass bioreactor. Dissolved CO_2 was monitored using a CO_2NTROL RS485 225 inline optical probe (Hamilton Bonaduz AG) connected to the Biostat® B-DCU control tower (analog port D, 4 – 20 mA, 0 – 100% relative solubility, 24 VDC), calibrated with 20% CO_2/N_2 test gas (MTI IndustrieGase AG) at 1021 hPa.

The reactor was inoculated at 1×10^6 cells/mL in 1 L HyClone™ SFM4CHO (4 g/L glucose, 2 mM glutamine). The process began in batch mode at 36 °C and 110 rpm, increasing to 120 rpm upon activation of the dCO_2 control cascade to enhance gas distribution.

Feeding Strategy

Daily bolus feeds of 100 mM glutamine stock and main feed (11.7 g/L HyClone™ CellBoost™ 6 supplement, 8.93 g/L HyClone™ SFM4CHO, 10.7 g/L glucose, 2.2 g/L sodium bicarbonate, 0.66 g/L Pluronic F68) started at 21 h and 44.5 h post-inoculation, respectively, based on integral viable cells (IVC) and glucose consumption rates (qGlc). Target concentrations were 3 ± 1 g/L glucose and 1.5 ± 0.5 mM glutamine. pH was set at 7.1 (deadband 0.1), adjusted to 7.0 post-dCO_2 control activation. Foam formation was suppressed by the addition of 2% antifoam solution (Dow Chemical Company).

Process Control

Dissolved oxygen (DO) was maintained at 60% saturation via a ring-sparger (0.8 mm orifice size) with air, O_2, and N_2 gas flows (max 0.075 L/min, up to 53% O_2 enrichment). The pH control initially used CO_2 and 1 M NaOH, with CO_2 reassigned to the dCO_2 controller after activation. The dCO_2 control cascade, implemented via a proportional-integral-derivative (PID) polygon controller, adjusted CO_2 (max 0.04 L/min) and air flows to maintain CO_2 concentrations corresponding to 10% (76 mm Hg) and 15% (114 mm Hg) of the maximum CO_2 solubility. Process data were logged using BioPAT® MFCS/win 3.1.

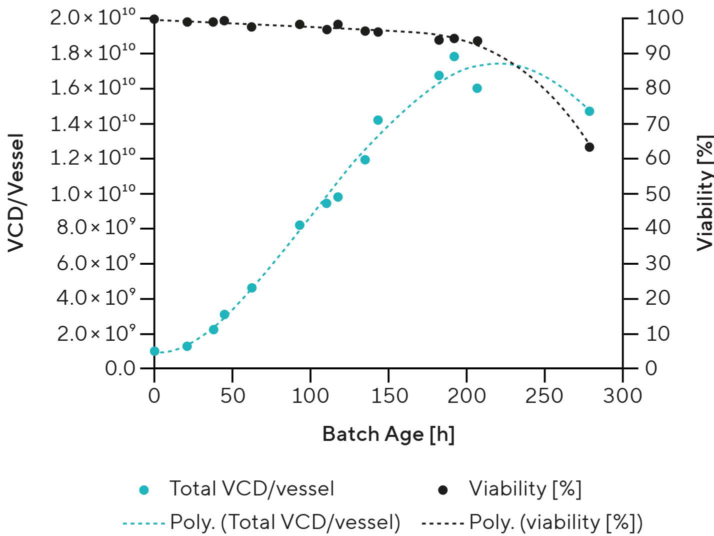
Analytics

Cell concentration and viability were measured with an Innovatis Cedex® AS 20 analyzer (trypan blue exclusion). Substrates (glucose, glutamine) and metabolites (NH_4^+, lactate, LDH) were quantified using a KoneLab™ Arena 20XT analyzer (Thermo Fisher Scientific). IgG was analyzed via precipitation-based turbidity assay (polyclonal pig anti-human IgG serum, Fisher Scientific) and Protein A HPLC (POROS™ A 20 µm column, Thermo Fisher Scientific). Osmolarity was measured with an OSMOMAT® 030 cryoscopic osmometer (Gonotec GmbH).

Results

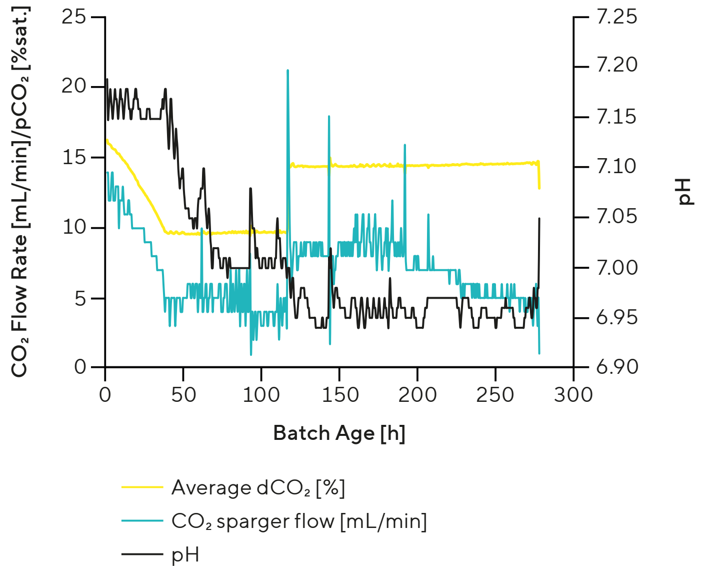
The process initiated at 1×10^9 cells/vessel (1×10^6 cells/mL, 99.7% viability) at 36 °C and 110 rpm, achieving a doubling time of < 24 h post-lag phase. Peak viable cell density (VCD) reached 9.5×10^6 cells/mL on day 7 with > 93% viability (Figure 1). A temperature shift to 34 °C halted growth, boosting IgG titer to 780 mg/L by day 9, when the process was terminated due to declining viability and maximum working volume limitations.

Figure 1: *Viable Cells and Viability of CHO Cultures During the Process*



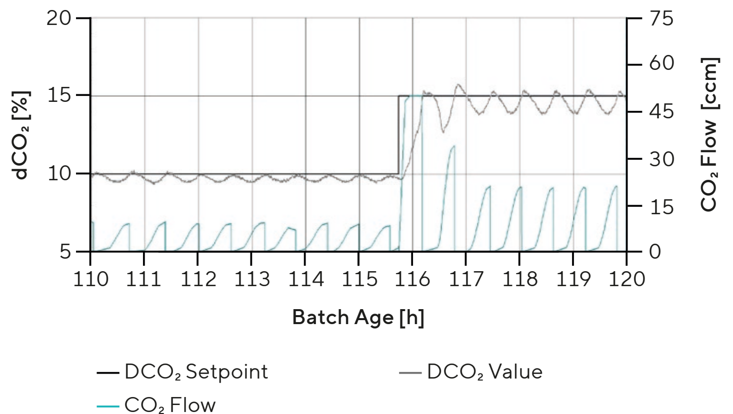
Initial dCO_2 exceeded 10% saturation due to pH stabilization requirements. Activation of the dCO_2 control at 38.3 h (VCD 2.4×10^6 cells/mL, 98.9% viability) maintained 10% saturation (76 mm Hg) for 79 h, followed by a step to 15% (114 mm Hg) at 117 h (VCD 7.6×10^6 cells/mL, 98.3% viability). The control strategy, enabled by the Biostat® B-DCU's PID polygon controller, ensured stable dCO_2 with minimal pH interference, despite bolus feeds and setpoint changes (Figure 2). Oscillations observed during the 10–15% setpoint step change (cycles of 35 min; Figure 3) indicate room for PID optimization in future Biostat® B-DCU software updates to enhance control stability and damping.

Figure 2: *Process Plot of dCO_2 with Respective CO_2 Sparger Flow Rates and Average pH*



Note. dCO_2 control was initiated at 38 h (10% saturation) and adjusted to 15% saturation at 117 h.

Figure 3: *Raw dCO_2 Values During a Setpoint Jump From 10% to 15% and Respective CO_2 Gas Flow Rates*



Cell division rate averaged 0.02 h^{-1} at 10% dCO_2, dropping to 0.008 h^{-1} at 15% dCO_2, representing a 127% increase in doubling time, and stopped completely after the temperature was lowered to 34 °C. Viability remained > 94%, indicating acceptable production conditions. A hypoxic event (pO_2 dropping to 1.6% saturation) due to limited O_2 flow (40 mL/min) was resolved by increasing the flow rate to 75 mL/min, restoring pO_2 control without compromising cell viability. This stress decreased lactate formation significantly, while glucose consumption remained stable (~ 3 g/L throughout process). It also boosted cell specific IgG productivity up to 9.9 pg/cell/day (>92 h), suggesting metabolic triggering. Consequently, the volumetric productivity was stabilized to an almost constant titer increase (Figure 4), despite higher dCO2 levels applied.

Figure 4: *Lactate Concentration and IgG Production (Titer)*

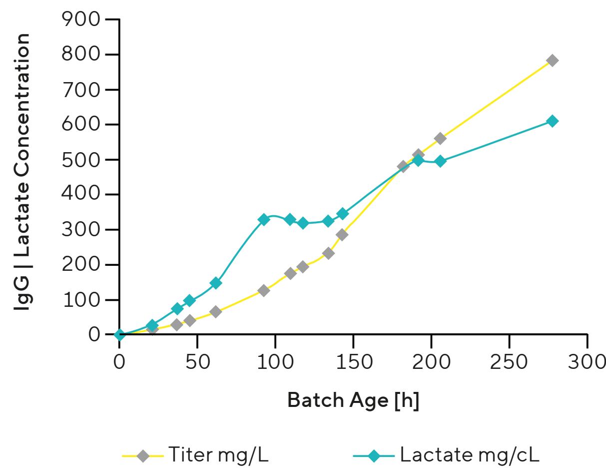
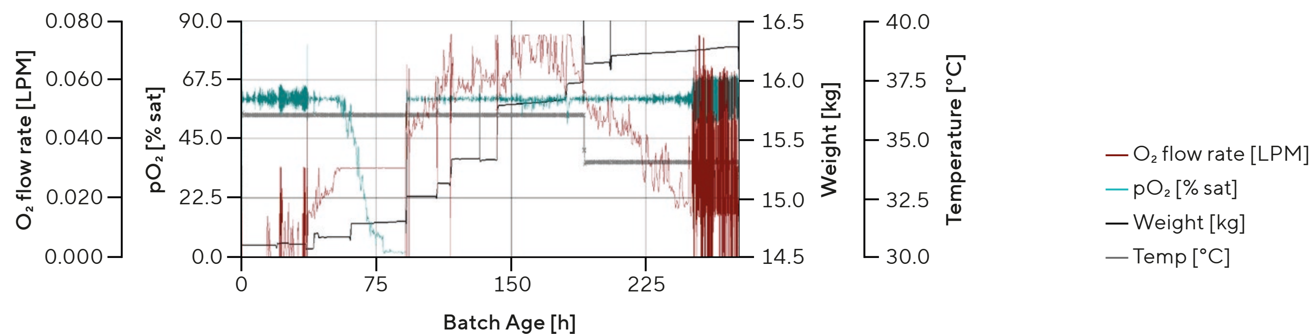


Figure 5 displays the profiles for vessel weight (filling level), temperature, pO_2, and O_2 gas flow rates throughout the fed-batch process. These results illustrate the Biostat® B-DCU's ability to maintain critical process parameters and recover rapidly from process disturbances like hypoxic stress.

Figure 5: *Filling Level (Weight), Temperature, and pO_2 With Corresponding O_2 Flow Rates*



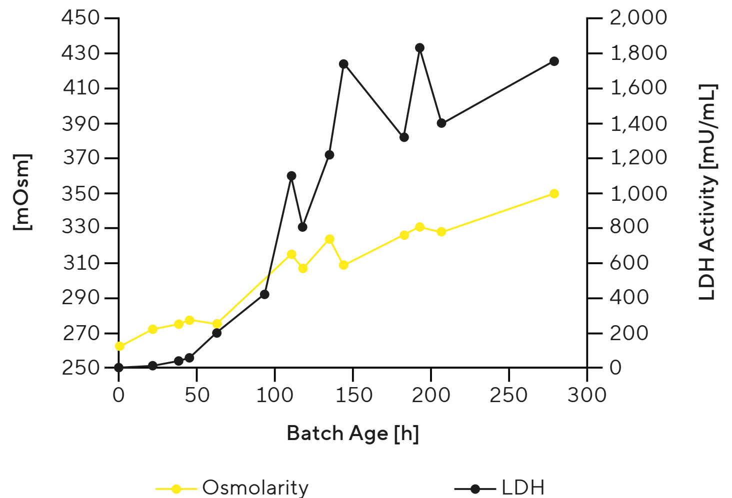
Note. Axis ranges for temperature and O_2 flow rate were 30 – 40 °C and 0 – 80 mL/min, respectively.

Final feeding steps caused lactate levels to again rise to 6.1 g/L at harvest, a risk of increased base consumption which could not be prevented by the temperature shift. To mitigate the risk of cell lysis associated with increased NaOH addition in response to elevated dCO_2 and lactate concentrations, the pH control deadband was expanded, and the upper pH limit was shifted to 7.1 during dCO_2 accumulation.

Osmolarity increased from 270 to 350 mOsm by the end of the process, a level considered critical, due to bolus feeding and a total NaOH consumption of 198 mL.

The onset of cell lysis was accompanied by an increase in lactate dehydrogenase (LDH) activity to 1.765 mU/mL in the culture medium, following the course of the osmolarity slope. The rise in LDH started after the activation of the dCO_2 control at 10% and was more pronounced after increasing the dCO_2 concentration to 15% after day 5, as shown in Figure 6. Ammonia levels remained stable at ~ 4 mM, minimizing cytotoxic effects.

Figure 6: *Osmolarity Increase by pH Titration with Base (NaOH) and LDH Activity as an Indicator of Cell Lysis*



Conclusion

The Biostat® B-DCU delivers precise, reliable dCO_2 control, enabling bioprocess engineers to optimize CHO-based IgG production. This study demonstrates the system's ability to maintain stable dCO_2 levels (10 – 15% saturation) with minimal impact on pH and DO control, achieving robust cell growth (peak VCD 9.5×10^6 cells/mL) and high titers (780 mg/L).

The integration of the CO_2NTROL probe into the Sartorius Automation System ensures real-time process monitoring and control, enabling the precise replication of large-scale stress conditions at bench scale. While moderate dCO_2 levels (10%) supported growth, higher levels (15%) reduced proliferation but did not limit productivity; hypoxic stress unexpectedly enhanced IgG output. Future PID refinements will further improve control stability. The Biostat® B-DCU's advanced automation and scalability make it an indispensable platform for developing efficient, high-yield bioprocesses.

References

1. Oliveira, J. E., Damiani, R., Vorauer-Uhl, K., Bartolini, P., & Ribela, M. T. (2008). Influence of a reduced CO_2 environment on the secretion yield, potency and N-glycan structures of recombinant thyrotropin from CHO cells. *Molecular biotechnology*, 39(2), 159 – 166. https://doi.org/10.1007/s12033-008-9047-6

2. Theeraseematham, P., Yamano-Adachi, N., & Omasa, T. (2024). *Effect of elevated carbon dioxide concentration on IgG production using CHL-YN cells* [Conference poster]. SCEJ 89th Annual Meeting. https://kuhner.com/wAssets/docs/download/Poster/Poster-NRC/SCEJ-meeting-Poster.pdf

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